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Ascending 5-HT Pathways and Behavioural Habituation

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DEAKIN, J. F. W., S. E. FILE, J. R. G. HYDE AND N. K. MACLEOD. *Ascending 5-HT pathways and behavioural habituation.* PHARMAC. BIOCHEM. BEHAV. 10(5)687–694, 1979.—Microinjections of 5,7 -dihydroxytryptamine into both the dorsal and median raphe nuclei resulted in 70–85% depletions in striatal and hippocampal 5-HT concentrations but did not affect habituation of orienting in the lick-distraction test, habituation of activity in an open-field or habituation of exploration in the holeboard test. Lesioned animals were hypoactive in the latter two tests and defaecated more than control rats in the open-field suggesting an increase in emotionality or fear. Rats with selective 5,7 -dihydroxtryptamine lesions of either the dorsal or the median raphe nucleus also showed no impairment of habituation of orienting or exploration. However, median raphe lesioned animals were hyperactive at some stages of the holeboard test. In contrast to previous studies, the results suggest intact ascending 5-HT pathways are not necessary for behavioural habituation of orienting, activity or exploration. Rather, 5-HT neurones may be involved in modulation of activity or responsiveness to aversive environments.

5-Hydroxytryptamine Raphe nucleus Habituation Orienting Exploration

IT HAS been suggested that serotonergic pathways may be involved in the process of behavioural habituation. For example, Carlton and Advokat [3] reported that administration of parachlorophenylalanine (PCPA, an inhibitor of 5-HT synthesis) to rats impaired habituation of a startle response. However, it is possible that PCPA administration alters the startle response itself, rather that disrupting the process of habituation. Conner, Stolk, Barchas and Levine [4] found that PCPA reduced the amplitude of a startle response to the first few stimulus presentations, but that after day 1, when the startle response had returned to normal levels, there was normal habituation. Fechter [7] also reported normal habituation of startle responses, following administration of PCPA. The evidence from electrolytic lesions is no more encouraging for the importance of 5-HT pathways in startle habituation. Davis and Sheard [6] found normal habituation following electrolytic lesions of the midbrain raphe nuclei but observed enhanced sensitization in their lesioned animals and therefore suggested that depletions of 5-HT might enhance sensitization, a process that is usually considered to be independent of habituation [15]. The finding that PCPA treatment resulted in enhanced dishabituation but normal habituation of an orienting response [9], is consistent with this suggestion.

However, Williams, Hamilton and Carlton [28] suggested that different pharmacological mechanisms might underlie habituation of different response systems. File [10] reported that PCPA disrupted within-session habituation of exploration, measured by the number of head-dips and the time spent head-dipping in a holeboard. Fibiger and Campbell [8] also found that PCPA impaired within-session habituation of locomotor activity in a novel environment.

Whilst the use of electrolytic lesions enables the function of specific pathways to be studied, such lesions have the disadvantage that they will also disrupt non-serotonergic fibres of passage. PCPA suffers from the twin disadvantages that it lacks neuroanatomical specificity and depletes 5-HT in all brain areas, and that it reduces brain noradrenaline levels [21].

The technique of lesioning selected 5-HT pathways with intracerebral microinjections of the specific neurotoxin 5, 7-dihydroxytryptamine (5,7-DHT) overcomes the disadvantages of electrolytic lesions and of PCPA. Using this technique Hole *et al.* [16] recently reported that 5, 7-DHT lesions of ascending 5-HT neurones impaired habituation of responses to touch and to tones. However, the method of rating responses may not have distinguished between a general increase in response amplitude and impaired habituation.

We have re-examined the role of 5-HT pathways in behavioural habituation, by selectively destroying 5-HT neurones in the midbrain raphe nuclei (B7 and B8) with microinjections of the neurotoxin 5,7-DHT. Habituation was studied in three behavioural situations: habituation of orienting, using a lick-distration task [11]; habituation of locomotor activity in the open field; and habituation of exploration in the holeboard [13]. The latter test was selected for a study of the effects of separate lesion of the dorsal and median raphe nuclei (B7 and B8 5-HT cell body groups respectively).

Apparatus

Distraction to auditory stimuli. The test chamber (19×19 × 26.5 cm) was enclosed in an acoustically-insulated box. A slit in the end wall gave access to a water spout and a drinkometer recorded each lick. Experimental events were automatically programmed and tones were delivered via a

loudspeaker placed in the lid of the chamber at the water spout end.

Open-field test. The open-field consisted of a square arena 61 cm × 61 cm, brightly illuminated by a 100 Watt bulb producing a light intensity of 1042 scotopic lux. The open-field was divided into four equal squares and was surrounded by a hardboard wall 25 cm high.

Exploration. The holeboard was a wooden box 45 × 55 × 55 cm, with four equally spaced holes in the floor, 3.8 cm in diameter. Objects could be placed 2 cm below these holes. The level of illuminance was 13 scotopic lux. Infrared photocells placed just under the holes provided an automated measure of the number of head-dips and of the time spent head-dipping. Cells in the walls of the box provided an automated measure of motor activity.

Surgery and Lesions

Animals were anaesthetized with tribromoethanol (250 mg/kg) or sodium pentobarbitone (40 mg/kg). Male hooded rats weighing 170–220 g were used throughout. 5,7-Dihydroxytryptamine lesions of dorsal (B7) and median (B8) raphe nuclei were made by microinjecting 9 μg of the neurotoxin dissolved in 3 μl of 0.02% ascorbic acid in saline vehicle through a cannula of 0.4 mm external diameter at a rate of 1 μl per min. The location of the cannula tip in the dorsal and median raphe nucleus was confirmed using dye injections. For separate lesions of B7 and B8 small injection volumes were used viz. 4 μg 5,7-DHT in 1 μl ascorbic acid in saline vehicle injected at a rate of 0.2 μl/min. Vehicle injected control animals were treated identically to 5,7-DHT lesioned animals except that only the vehicle was microinjected.

It was thought possible that some of the behavioural effects of 5,7-DHT could have been due to non-specific damage to catecholamine cell bodies and fibres known to be present in the raphe nuclei. Therefore, a further group of animals received microinjections of 6-hydroxydopamine (6-OHDA) into the same B7 and B8 sites, in the same volume of vehicle and at the same rate as 5,7-DHT lesioned animals. The quantities of 6-hydroxydopamine injected were: combined B7 + B8 lesions 8 μg in 3 μl 0.02% ascorbic acid in saline vehicle, separate B7 or B8 lesions 4 μg in 1 μl vehicle. A vehicle injected control group for the 6-OHDA lesions was prepared. These animals were treated identically to 6-OHDA lesioned animals except that only the 0.02% ascorbic acid in saline vehicle was microinjected. Habituation of orienting and of activity were carried out 10–14 days postoperatively and open field testing in the fifth postoperative week.

Procedure

Habituation of orienting. Eight lesioned rats (combined B7 and B8) and eight vehicle injected controls were water deprived for 48 hours and thereafter received daily water in the test chamber and immediately after testing. (Because of possible damage to the ear-drums caused by the ear bars only rats showing a response to a click were used in this study.)

On the first day in the test chamber each rat was given 20 mins with free access to the water spout and without any tone presentations. On the second day each rat's 200th lick turned on a control period of 9 sec during which the number of licks (A) was counted. The next 20th lick turned on a 9 sec tone (7KHz, 85 dB) and the number of licks made during the tone presentation (B) was counted. A distraction ratio of (A-B)/A was calculated; the ratio is zero if there is no distraction at all to the tone, (i.e A=B) and one if there is total distraction (i.e B=O). Tones were presented until 3 successive tone presentations produced ratios of less than 0.10 In each group one rat failed to reach this criterion on the first day of tone presentations and these rats were given a second day of tone presentations. The day after reaching habituation criterion the rats were returned to the test chamber and given a 24-hr retention test. Any rat not showing complete retention was once more taken up to the habituation criterion. The

TABLE 1

NUMBERS OF RATS IN DIFFERENT EXPERIMENTAL GROUPS IN HOLEBOARD EXPERIMENT

			Holeboard	
			Objects Present	Objects Absent
A)	Combined B7 + B8 lesions and controls		3 days	1 day
	a) 5,7-DHT	B7 + B8	12	12
	Vehicle	B7 + B8	12	12
	b) 6-OHDA	B7 + B8	10	—
	Vehicle	B7 + B8	7	—
B)	Separate B7 and B8 lesions and controls		2 days	1 day
	a) 5,7-DHT	B7	12	12
	5,7-DHT	B8	12	12
	Vehicle	B7 or B8	10	10
	b) 6-OHDA	B7	—	11
	6-OHDA	B8	—	12
	Vehicle	B7 or B8	—	14

rats received one further retention test 2 weeks later.

Habituation of open-field activity. Ten lesioned rats (combined B7 and B8), 9 vehicle injected rats and 10 6-hydroxydopamine injected rats were tested in the open-field. All animals had been well handled but not exposed to the open-field. Each animal was tested in the open-field for 5 min and activity recorded as the number of squares entered per minute; in addition, the number of rears on to the hind legs and the number of stools were recorded. Urination was rated on a scale from 0 through 1 (less than 2 drops urine) to 3 (pool of urine greater than 2 cm in diameter). All observations were carried out without knowledge of the experimental group to which each rat belonged.

Habituation of exploration. Rats were tested in random order between 0800 and 1200 hours. Each rat was placed in the holeboard with objects present under the holes for 10 mins and the number of head-dips made, the time spent head-dipping and motor activity were automatically recorded. The rats were tested for 2 or 3 consecutive days, in order to study between-day habituation, and were then tested for retention 1 week later.

Since the effects of drugs on exploration have sometimes been found only when rats are tested in the holeboard in the absence of objects [10], some further groups were given one day's test in the holeboard without any objects under the holes. The main comparisons were between 5,7-DHT lesioned and vehicle control animals. Where significant 5, 7-DHT lesion effects were obtained, the experiment was repeated with a new set of vehicle injected control animals and the appropriate 6-OHDA injected group. Thus, the possibility that a 5,7-DHT lesion effect was due to non-specific damage to catecholamine neurones could be directly evaluated. The experimental conditions are summarized in Table 1.

5-HT Assay

At the completion of testing, rats were stunned and rapidly decapitated. Brain areas were dissected out on a cold petri-dish. 5-HT depletions were measured in the corpus striatum and hippocampus which are respectively the main projection areas of the dorsal and median raphe nucleus. 5-HT was assayed by the method of Curzon and Green [5].

RESULTS

Habituation of Orienting

Table 2 shows the mean distraction ratios to the first tone presentation for the control and lesioned rats, the trials to habituate and the trials to rehabituate 2 weeks later. There was no significant difference between the lesioned and the control animals in their distraction to the first tone presentation ($t(14)=0.92$) or in their rate of habituation ($t(14)=0.73$). On the 24 hr retention test only one rat in each group failed to show complete retention and required 2 further tone presentations in order to regain habituation criterion. Control and lesioned rats showed equal and significant retention when tested 2 weeks later.

Habituation of Open-field Activity

5,7-DHT lesioned and vehicle injected control animals showed significant habituation of activity over the 5 min test period, $F(4,64)=7.58$, $p<0.001$. There was no significant lesion × trial period interaction indicating that the 5,7-DHT treatment did not affect habituation of locomotor activity (Fig. 1). 5,7-DHT lesioned animals reared significantly less than vehicle injected control rats, $F(1,17)=5.42$ $p<0.05$. 5, 7-DHT lesioned animals urinated significantly more than vehicle control animals ($p<0.1$) and 6-OHDA injected controls ($p<0.01$). Defecation was also increased in 5,7-DHT lesioned animals compared to vehicle controls ($p<0.05$) but not significantly compared to 6-OHDA injected animals (Fig. 1).

TABLE 2

MEAN(± SEM) DISTRACTION RATIO TO INITIAL TONE PRESENTATION TRIALS TO HABITUATE AND TRIALS TO REHABITUATE 2 WEEKS LATER

	Initial Distraction Ratio	Trials to Habituate	Trials to Rehabituate
Controls	0.42 ± 0.11	8.8 ± 1.7	2.5 ± 1.4
Lesioned (B7 +B8)	0.28 ± 0.09	7.1 ± 2.1	2.7 ± 0.6

Habituation of Exploration

Statistics. The data were subjected to split plot analysis of variance in which the lesion was the between subjects factor, and days and trial periods were within subjects factors. The scores for each 10 min trial were broken into four 2.5 min periods to give a measure of within-session habituation. A lesion-induced impairment of habituation would be reflected in a significant lesion×trial interaction for within-session habituation and lesion×day interaction for between-day habituation.

Combined Lesions of B7 and B8.

5,7-DHT lesioned rats tested with objects present showed significant within-session habituation of exploration, duration of head-dipping: $F(3,63)=30.32$, $p<0.001$ and number of head dips: $F=10.15$, $p<0.001$ and there was no significant lesion × trial period interaction indicating the lesion had not affected within-session habituation (Fig. 2). Similarly both groups showed significant between-day habituation of exploration and there was no lesion effect. Motor activity in 5,7-DHT lesioned rats also showed significant within-session and between-day habituation, $F(3,63)=50.5$, $p<0.001$ and $F(2,42)=3.24$, $p<0.05$ respectively.

Although the 5,7-DHT lesions did not affect habituation, the level of exploration was reduced in lesioned animals, duration head-dipping: $F(1,21)=5.13$, $p<0.05$ and number of head dips: $F(1,21)=4.0$, $p<0.1$, and activity scores were also reduced, $F(1,21)=15.77$, $p<0.001$, as shown in Fig. 3. These reductions in exploration and activity cannot be attributed to catecholamine depletion since the rats injected with 6-OHDA did not differ significantly from their vehicle injected controls.

Both 5,7-DHT lesioned and control rats showed evidence of retention when tested in the holeboard one week later (see Fig. 2) and in neither case was there a significant increase in the number of head dips made or in the time spent head-dipping.

Tested in the absence of objects, the 5,7-DHT lesioned

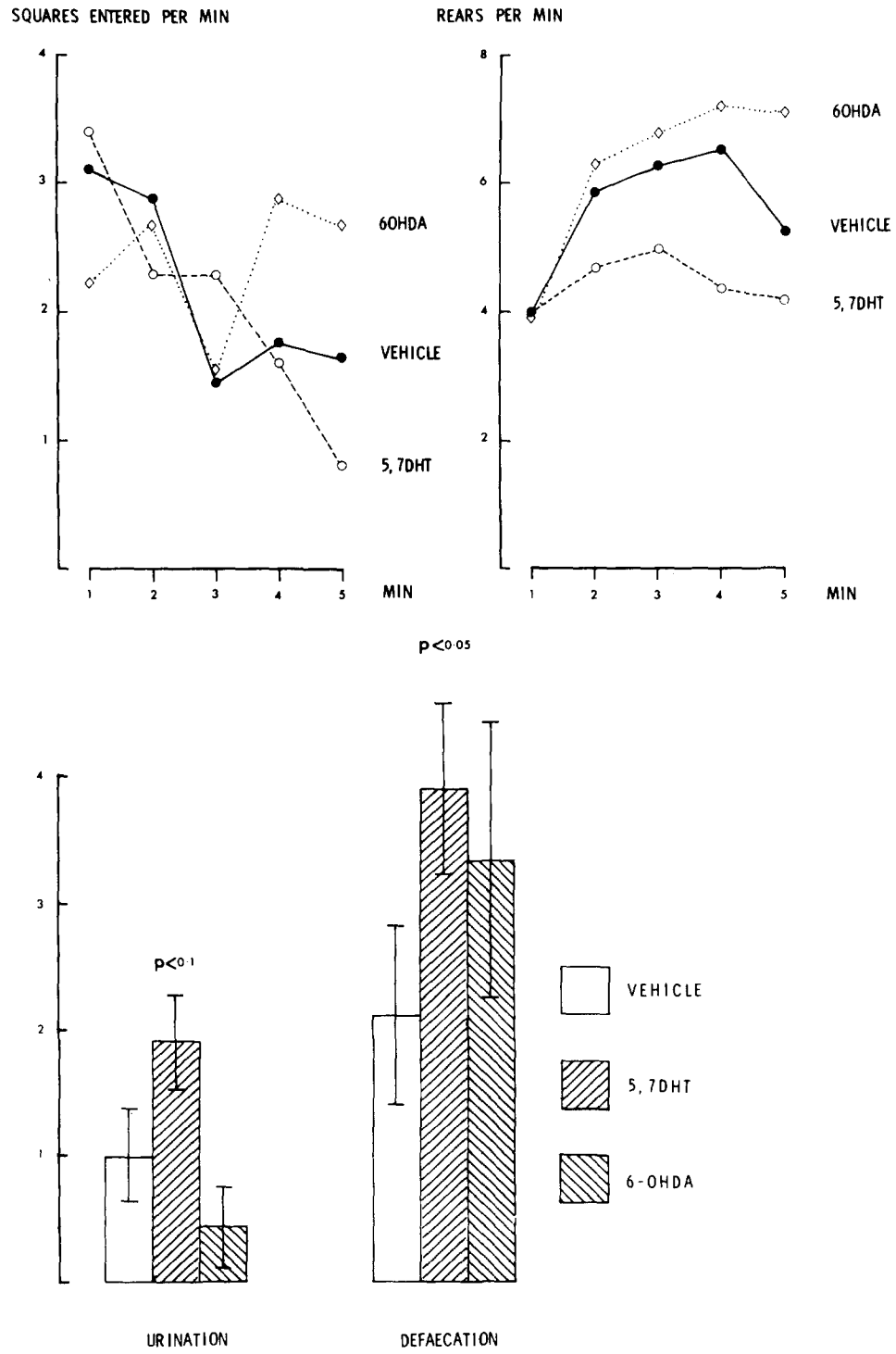

FIG. 1. Effects of 5,7-DHT, vehicle or 6-OHDA microinjections in the open-field test. Vertical bars =S.E.M. For defaecation vertical axis represents mean number of stools deposited during the 5 min test, and for urination the mean urination rating (see methods). Significance levels assessed by *t*-test for urination and defaecation.

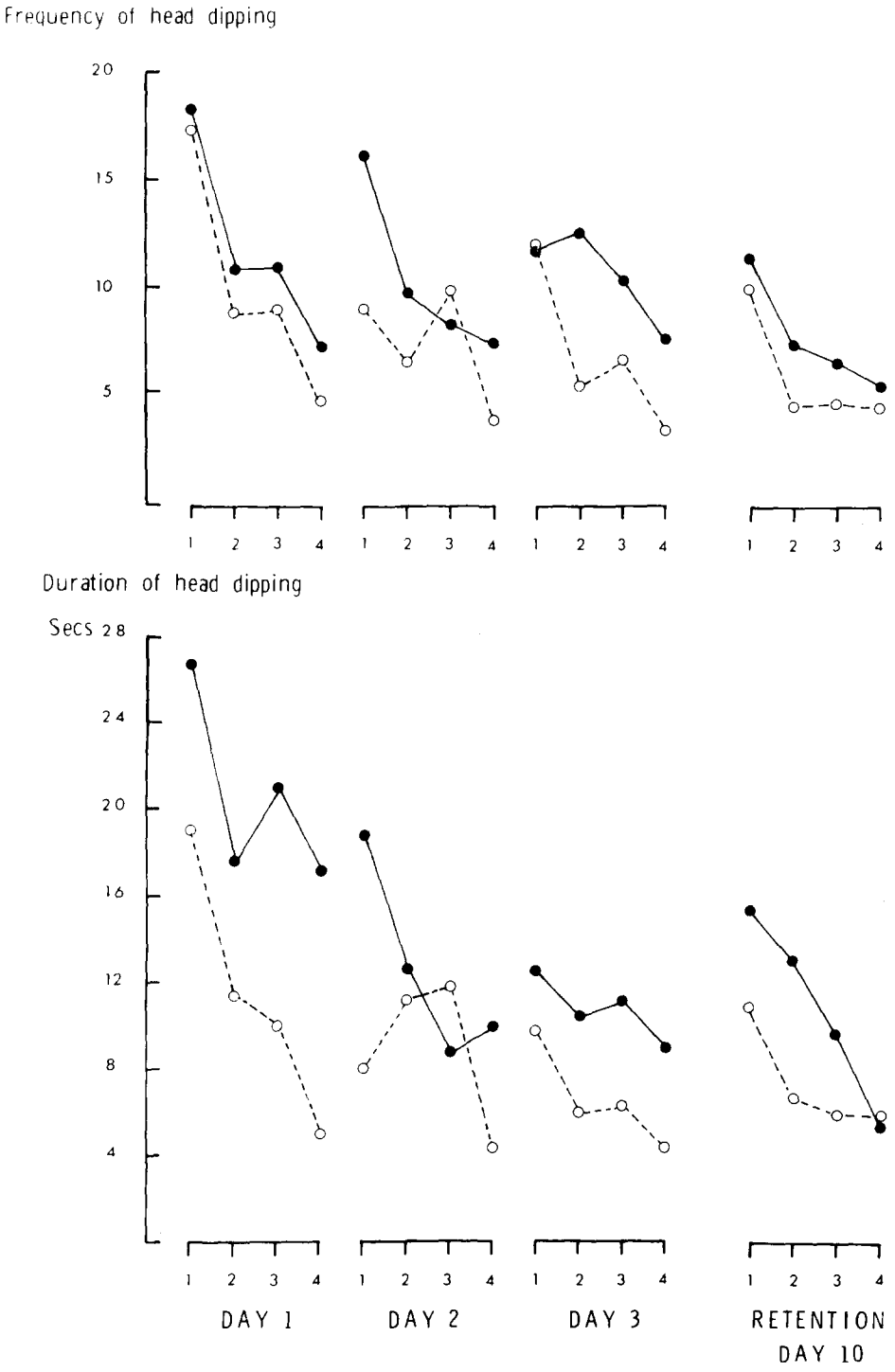

FIG. 2. Habituation of exploration in the holeboard with objects present. Vehicle injected; ●——●; and 5,7-DHT injected ○——○ animals. Animals were tested for 10 min on three successive days and tested for retention 10 days after the first test. Results plotted at successive 2.5 min intervals of each session.

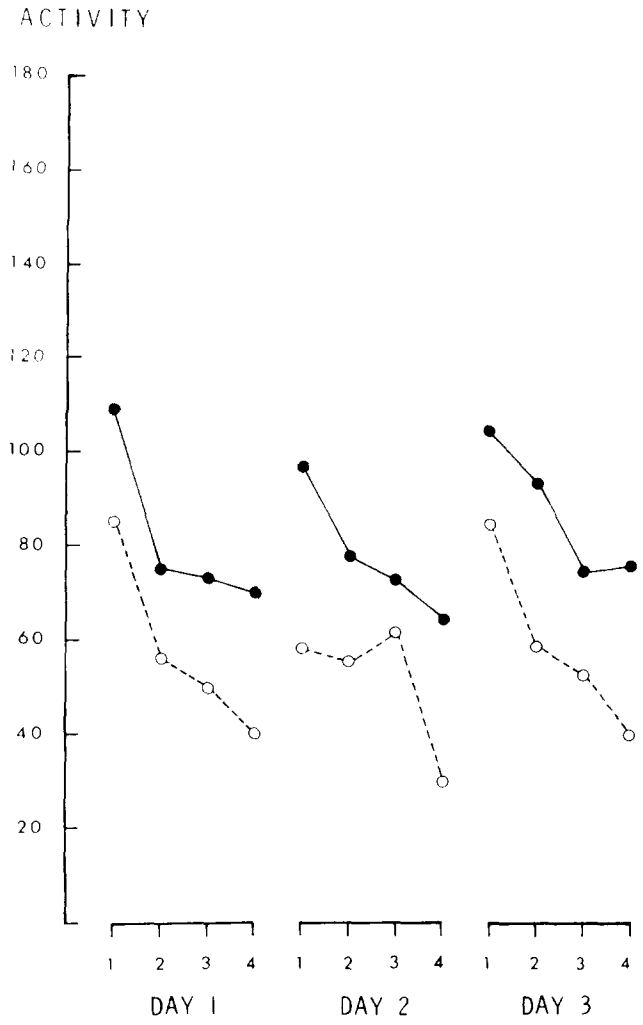

FIG. 3. Activity of vehicle (●——●) and 5,7-DHT (○——○) injected animals in the holeboard in the presence of objects. Results plotted for successive 2.5 min of 10 min test period on each of three successive days.

rats did not show significant reductions in exploration and motor activity. Once more there was significant habituation, $F(3,66)=4.62$, $p<0.01$ for number of head dips, $F=3.62$, $p<0.025$ for time spent head-dipping and $F=3.10$, $p<0.05$ for activity, and no evidence of any lesion-induced impairment in habituation.

Lesions of B7

The data from one lesioned rat were excluded because it did not have significant depletion of striatal 5-HT. There was no difference between the B7 and B8 vehicle injected controls and therefore their results were combined.

When the rats were tested in the presence of objects the rats with lesions of B7 did not show significant differences in the number of head dips or in the time spent head-dipping. All the rats showed significant within-session habituation, $F(3,57)=6.25$ for number of head dips and $F=68.0$, $p<0.001$ for time spent head-dipping.

Figure 4 shows the mean activity scores for Days 1 and 2, with the scores from each session divided into four 2.5 min periods. Both lesioned and control rats showed significant within-session habituation, $F(1,19)=18.8$, $p<0.001$, but the decrease was more marked for the B7 group, giving rise to a significant lesion × trial interaction, $F(1,19)=4.6$, $p<0.05$.

The groups of rats tested without any objects in the holeboard showed a similar pattern of results. The group with B7 lesions did not differ from the controls in the number of head dips made or in the time spent head-dipping and both groups showed significant within-session habituation, $F(3,57)=10.1$, $p<0.001$ for number of head dips; $F(3,57)=5.22$, $p<0.01$ for the time spent head-dipping. Both groups also showed significant habituation of motor activity, $F(3,57)=15.7$, $p<0.001$. Unlike the effect of combined lesions, rats with lesions of B7 showed only slight hypoactivity which was of borderline significance, $F(1,19)=3.9$, $p<0.06$.

Rats injected with 6-OHDA into B7 and vehicle-injected controls were also given a 10 min test in the absence of objects. There were no differences in the number of head dips or in the time spent head-dipping and both showed significant habituation, $Fs(1,16)=5.6$, $p<0.01$ and $=4.5$, $p<0.05$ respectively. Motor activity also showed significant habituation, $F(1,16)=19.7$, $p<0.001$. The 6-OHDA injected rats did not differ in their level of motor activity from the vehicle-injected controls, and thus the slight hypoactivity seen in the B7 5,7-DHT lesioned group cannot be attributed to damage to catecholamine neurones.

Lesions of B8

Three 5,7-DHT lesioned animals were discarded from the analysis as they had inadequate hippocampal 5-HT depletions. When the rats were tested with objects present in the holeboard those with 5,7-DHT lesions of B8 did not differ

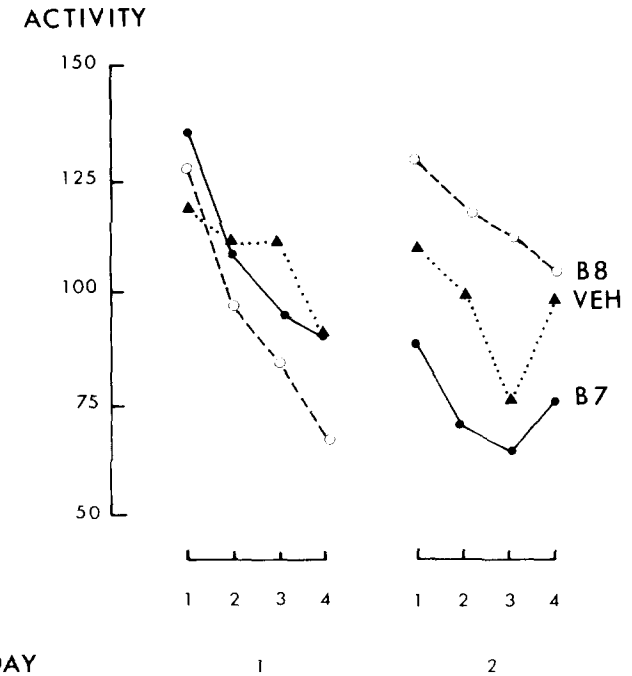

FIG. 4. Effects of selective B7 and B8 5,7-DHT lesions on activity in the holeboard test with objects present. Results plotted as 10 min test sessions divided into 4, 2.5 min epochs. Rats were tested on two successive days.

from controls in either the number of head dips made, nor in the time spent head-dipping. Both lesioned rats and controls showed significant within-session habituation, $F(3,51)=3.2$, $p<0.05$ for number of head dips; $F(3,51)=4.5$, $p<0.01$ for time spent head-dipping, and significant between-day habituation, $F(1,17)=12.5$, $p<0.01$ for number of head dips; $F(1,17)=12.8$, $p<0.01$ for time spent head-dipping. There was no significant lesion × trial period or lesion × day interaction.

Activity scores of both the 5,7-DHT lesioned and the control group showed significant within-session habituation, $F(3,51)=10.2$, $p<0.001$, but the 5,7-DHT B8 lesioned rats did not show between-day habituation of activity (Fig. 4). This resulted in a significant lesion × day interaction, $F(1,17)=9.9$, $p<0.01$. Figure 4 shows that motor activity in the 5,7-DHT B8 group increased on Day 2, rather than showing a decrease and that they remained hyperactive throughout the session.

Another set of 5,7-DHT B8 lesioned rats and controls were tested in the absence of objects for a single 10 min session. No lesion effect on the level of exploration or on habituation of exploration was observed. However, 5,7-DHT lesioned rats had significantly greater motor activity scores than controls, $F(1,19)=7.6$, $p<0.05$, and these rats showed a tendency to impaired habituation of motor activity which just failed to reach significance, $F(3,57)=2.6$, $p<0.06$. The hyperactivity observed in the absence of objects was not due to damage to catecholamine neurones since rats injected with 6-OHDA were not hyperactive in comparison with their vehicle injected controls, $F(1,17)=3.4$.

Biochemical Confirmation of Lesions

Figure 5 demonstrates that 5-HT concentrations were greatly reduced in the corpus striatum and hippocampus of the combined B7+B8 5,7-DHT lesioned animals. Striatal 5-HT terminals originate almost entirely from the dorsal raphe nucleus (B7) [18] and selective 5,7-DHT lesions of B7 produced a fall in striatal 5-HT concentrations. However, this was not as marked as in the combined B7+B8 lesioned group and was accompanied by a decrease in hippocampal 5-HT concentrations. This may have been due to spread of the neurotoxin to involve B8 cell bodies, the main source of hippocampal 5-HT [18] or to damage of caudal dorsal raphe cell bodies which also contribute to hippocampal 5-HT [19]. Selective 5,7-DHT lesions of the median raphe nucleus (B8) produced a reduction in hippocampal 5-HT levels which was not as marked as in the combined lesions; however, striatal 5-HT concentrations were not affected.

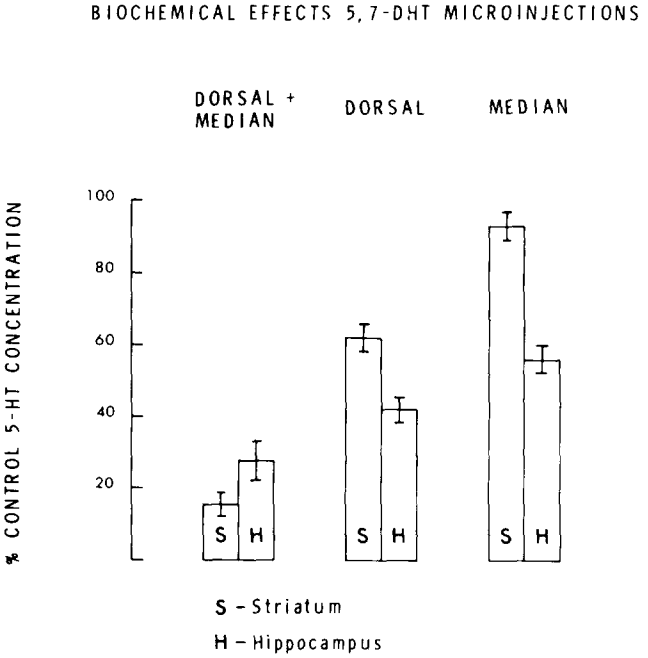

FIG. 5. Effects of 5,7-DHT lesions on 5-HT concentrations in striatum and hippocampus.

DISCUSSION

The results demonstrate that habituation of the orienting response to tones in the lick distraction test is not dependent on intact ascending serotonergic projections. This result is in agreement with the previously reported lack of effect of parachlorophenylalanine induced 5-HT depletion on habituation of lick distraction [9]. However, Hole *et al.* [16] reported that destruction of ascending 5-HT fibres with intracerebral microinjections of 5,7-DHT increased the number of auditory stimulus presentations necessary for habituation to a criterion to occur. Although the latter results may have been due to a lesion-induced increase in orienting responses, rather that impaired habituation, no increase in response magnitude in terms of duration was observed in the present study. Indeed, in the previous study it was observed that the initial lick distraction response was reduced in PCPA pre-treated animals [9]. Nevertheless, the present results do not exclude the possibility that the amplitude of the orienting response, but not its duration, may have been increased as a result of the 5,7-DHT lesions and that this may have produced the apparent impairment of habituation noted by Hole *et al.* [16].

5,7-DHT lesioned animals reared less and defaecated more than control animals (Fig. 1). This may indicate that the lesions had increased emotionality or fear in the open-field. However these results need to be reconciled with the 'anxiolytic' effects of PCPA and 5,7-DHT lesions using conflict tests [14,27]. Nevertheless the results suggest brain 5-HT neurones may be involved in locomotor and other reactions to aversive stimuli and further experiments using benzodiazepine sensitive models of anxiety are in progress to investigate this possibility [12]. Habituation of horizontal activity (squares entered per min) in the open-field was not affected by the lesions but this is likely to involve habituation of fear as well as exploration. The holeboard provides a specific measure of exploration and the 5,7-DHT lesions of B7 and B8 were without effect on habituation of exploratory head-dipping. The possibility that the combined B7+B8 5,7-DHT lesions may have masked an effect of destruction of either nucleus alone is excluded by the finding that more restricted injections of 5,7-DHT in B7 or B8 also had no effect on habituation of exploration in the holeboard. The results indicate that ascending 5-HT neurones are not involved in habituation of exploration and suggest that the previously reported PCPA induced habituation impairment in the holeboard test [10] was due to impaired serotonergic function in non-ascending 5-HT neurones or to non-specific actions of the drug.

A striking effect of electrolytic median raphe nucleus le-

sions is to produce a sustained hyperactivity [18, 22, 25]. However, since PCPA induced 5-HT depletion does not consistently produce hyperactivity [2, 8, 20, 26, 28] and since 5,7-DHT lesions in previous studies tended to produce hypoactivity [17,22] it has been argued that the effects of electrolytic lesions are not due to damage to 5-HT neurones. Our observation that 5,7-DHT lesions of B7+B8 reduced rearing in the open-field and activity in the holeboard might appear to support this contention. However, 5,7-DHT lesions of B8 5-HT neurones (median raphe nucleus), whose specificity was confirmed by a selective depletion in hippocampal but not striatal 5-HT, did produce greater activity scores at some stages of the holeboard test (Fig. 4) than either vehicle or 6-OHDA injected control animals. However, hippocampal 5-HT was reduced to an equal extent in the animals with B7 5,7-DHT microinjections yet hyperactivity was not observed. One possibility is that the reduction in striatal 5-HT concentrations in the B7 lesioned animals counteracts the effects of hippocampal 5-HT depletions. Alternatively, since caudal B7 neurones innervate different areas of the hippocampus from those innervated by B8 [1, 24] there may be functional differences between the two hippocampal projections. Although the hyperactivity observed in B8 5,7-DHT lesioned animals was neither marked nor persistently present, the finding suggests that damage to 5-HT neurones may be partly responsible for hyperactivity observed after electrolytic destruction of this nucleus.

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